

Female mating preference for bold males in the guppy, *Poecilia reticulata*

(boldness/predator inspection/fleeing/mate choice/fish)

JEAN-GUY J. GODIN* AND LEE ALAN DUGATKIN†

*Department of Biology, Mount Allison University, Sackville, NB E0A 3C0, Canada; and †Department of Biology, University of Louisville, Louisville, KY 40292

Communicated by Gordon H. Orians, University of Washington, Seattle, WA, July 11, 1996 (received for review March 25, 1996)

ABSTRACT Although females prefer to mate with brightly colored males in numerous species, the benefits accruing to such females are virtually unknown. According to one hypothesis of sexual selection theory, if the expression of costly preferred traits in males (such as conspicuous colors) is proportional to the male's overall quality or reveals his quality, a well-developed trait should indicate good condition and/or viability for example. A female choosing such a male would therefore stand to gain direct or indirect fitness benefits, or both. Among potential phenotypic indicators of an individual's quality are the amount and brightness of its carotenoid-based colors and its boldness, as measured by its willingness to risk approaching predators without being killed. Here, we show experimentally that in the Trinidadian guppy (*Poecilia reticulata*) the visual conspicuousness of the color pattern of males correlates positively with boldness toward, and with escape distance from, a cichlid fish predator. Bold individuals are thus more informed about nearby predators and more likely to survive encounters with them. Mate-choice experiments showed that females prefer colorful males as mates, but prefer bolder males irrespective of their coloration when given the opportunity to observe their behavior toward a potential fish predator. By preferentially mating with colorful males, female guppies are thus choosing on average, relatively bold, and perhaps more viable, individuals. In doing so, and to the extent that viability is heritable, they potentially gain indirect fitness benefits by producing more viable offspring than otherwise.

Although females prefer to mate with brightly colored males in numerous species, the benefits accruing to such females are virtually unknown (reviewed in refs. 1–5). According to one hypothesis (the “good-genes” model) of sexual selection theory, if the expression of costly preferred traits in males is proportional to the male's quality or reveals his quality (see ref. 4), a well-developed trait should indicate social dominance, good condition, good nutritional status, low parasite load, high behavioral vigor and/or high viability. A female choosing such a male would therefore stand to gain direct or indirect fitness benefits, or both (1–5).

Among potential phenotypic indicators of an individual's quality are the amount and brightness of its carotenoid-based colors (3, 4) and its boldness, as measured by its willingness to risk approaching predators without being killed (6–8). Body coloration (9–15) and boldness (6, 7) vary widely among individuals within populations. Maximum expression of either of these traits in males may be costly, at least in terms of increased risk of mortality to predation (6, 9, 16–18). Therefore, presumably, only high-quality individuals can develop and maintain bright color patterns and dare approach predators often and survive. Predator approach behavior has recently been shown to deter predator attack in fish (8). Bolder

individuals may thus be more viable than relatively timid ones. Variation among males in body coloration and boldness level provides females with potential proximate cues for making mate-choice decisions. However, the relative availability of such information to females in nature may be different for these two male traits. If coloration and boldness are positively correlated traits, females could thus be mating on average with relatively bold males by preferentially choosing colorful individuals. In doing so, and to the extent that viability is heritable, they may gain indirect fitness benefits by producing offspring with greater viability than otherwise. Such choosy females could also benefit directly (e.g., resources, higher level of paternal care) by mating with bold, colorful males. To our knowledge, no information exists on the relationship between a male's coloration and its boldness level, and on female mating preferences for bold males.

Here we report the results of experiments that investigated for the first time the relationships between the visual conspicuousness of the color pattern of individual males and their boldness toward, and escape potential from, a natural predator. In addition, we present the results of an experiment that quantified female preferences for both male coloration and boldness level separately. We used the Trinidadian guppy (*Poecilia reticulata*) as a suitable model species for the following reasons. The guppy is sexually dichromatic, with males being polymorphic in color owing to patches of carotenoid and melanin pigments and structural colors over their body (9, 16, 19). This species has a nonresource-based mating system, in which the mating success of males can be determined by female choice and females only gain sperm from them (20). Although fish predators apparently select against conspicuous male color patterns (9, 16), female mating preferences for colorful males are widespread among wild guppy populations (21, 22), are generally consistent within individuals (23), and have a heritable component (11, 21, 24). Body color pattern in male guppies similarly has heritable genetic variation (9, 24, 25), although the brightness of the carotenoid-based color patches in particular is dependent on their diet (9, 26) and parasite load (27, 28). An individual's quality may also be reflected in its level of boldness or willingness to take risks (6–8) and its ability to avoid predators (cf. ref. 16). Guppies take risks when they approach (“inspect”) potential predators at a distance, most commonly alone or in pairs, in a characteristic threat-sensitive manner (8, 29–31). Predator inspection behavior varies considerably between individuals in the guppy (32, 33), as in other fish species (reviewed in ref. 6), and such behavioral variation may have a genetic component (34–36). Bright color patterns and boldness in male guppies thus appear to be honest indicator traits of their quality, on which females may proximally base their mate-choice decisions.

METHODS AND RESULTS

General Methods. Experimental female guppies were first generation, laboratory-born descendants of wild guppies collected from the Quaré River, Trinidad, West Indies (10°41'25"N, 61°11'51"W; Trinidad National Grid PT9500,

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. §1734 solely to indicate this fact.

PS8085). All females were raised separately from males and were thus virgins when tested; virgin guppies are highly receptive to male courtship displays (20). Because naive female guppies may initially mate indiscriminately (11), our virgin females were allowed indirect experience with courting males by viewing them through the glass panes of adjacent holding aquaria prior to testing. Experimental males originated from the Quaré River. They were allowed to court and mate with other females (not used in this study) in their home aquaria prior to being used in the current study. Guppies in this population are exposed to relatively high fish predation pressure, occur in mixed-sex shoals, and have been observed to approach fish predators in the wild (37). To quantify boldness and escape potential in male guppies under predation hazard, we used either a live pike cichlid fish (*Crenicichla alta*) or a model of it as a predatory threat depending on the particular experiment. This cichlid is a natural predator of the guppy (9), and occurs in the Quaré River (37). Other known guppy predators in this river include the blue acara cichlid (*Aequidens pulcher*), the characin *Astyanax bimaculatus*, and the cyprinodont *Rivulus hartii* (J.-G.J.G., personal observations).

Fish holding aquaria contained a corner water filter, a plastic plant, and gravel substratum, and were maintained at 24–26°C under a 12 h light/12 h dark illumination cycle provided by Sun-Glo fluorescent tubes (R.C. Hagen, Montréal), which simulate the energy spectrum of sunlight. Guppies were fed *ad libitum* flake food (NutraFin) three times daily, supplemented with live brine shrimp nauplii.

Our study comprised four related laboratory experiments. No food was available to the fish during any of the experimental trials.

Experiment 1. This experiment tested the hypothesis that the boldness of brightly colored male guppies, measured as their frequency of approaches initiated toward a potential fish predator, is greater than that of less colorful individuals.

The experimental tank (100 cm × 25 cm, with a water level of 15 cm) comprised two compartments, a large one that contained the guppies and a smaller one (15 cm × 25 cm) that housed the cichlid predator (13 cm, standard length). These compartments were separated by two adjacent Plexiglas partitions, one clear and permanent and the other opaque and removable, and had a gravel substratum. The tank was illuminated overhead with a Sun-Glo fluorescent tube (24 μ E/m²/s at the water surface), and water temperature was maintained at 24–26°C. We quantified the level of boldness of paired bright and drab males in the presence and absence of the predator when females were present. As the predator and guppies were physically separated by a clear Plexiglas partition, no actual predatory attacks occurred. Males were tested in pairs to provide each male the choice of either initiating an inspection visit (the riskiest option), following the other male who initiated the approach, or not inspecting at all. Paired males were intentionally chosen from their home aquaria to differ in their overall color patterns, but otherwise matched for body length. The brighter male (mean \pm SE standard length = 18.7 \pm 0.5 mm) in a pair always possessed more and relatively larger, more saturated, and darker color patches than the drabber male (19.2 \pm 0.5 mm), and was thus more visually conspicuous (cf. ref. 39), at least to our eyes.

For a given trial, a preassigned pair of males and two nongravid (sexually receptive) adult females were placed into the larger compartment and allowed to acclimate for 2 h, during which time the opaque partition blocked their view into the predator compartment. Following this period, the opaque partition was raised remotely, allowing the guppies to view the predator compartment, which either contained the live cichlid or was empty. The number of approaches toward the predator (or the empty predator compartment in the tank) initiated separately by the bright and drab males was then recorded from behind a blind for 30 min. Fifteen different pairs of males

were similarly tested for each of the two predator treatments separately, and different females were used for each trial. Individual fish were thus used only once in the experiment.

When females were present nearby, the more brightly colored males approached the live fish predator significantly more often than the paired drab males (Fig. 1). However, in the absence of the predator, the levels of boldness (as measured by inspections of the empty predator compartment) of the paired males were similar, owing to a significant reduction in the inspection frequency of the more brightly colored males (Fig. 1). Differences in the boldness of paired males differing in coloration were therefore only realized when a threat of predation was imminent.

Experiment 2. Given the results of Experiment 1, and that guppies commonly occur in mixed-sex shoals (31), this corollary experiment ascertained whether the relative boldness level of paired male guppies toward a predator depends on their seeing a female nearby, and further tested the hypothesis that the boldness exhibited by individual male guppies in the presence of a female correlates positively with the visual conspicuousness of their respective body color pattern.

The experimental tank and lighting conditions were identical to the ones used in the first experiment, except that the live predator was replaced with a model of a pike cichlid to standardize the predatory threat and the free-swimming female guppies were replaced with one adult stimulus female located in a clear Plexiglas cylinder (8 cm diameter) at the opposite end of the tank from the predator model (in the female-present treatment only). An empty cylinder only was presented in the female-absent treatment. The predator model was constructed from body filler material using a mold made from a freshly sacrificed pike cichlid (14 cm). Glass eyes were fixed into the model, which was painted realistically and coated thinly with fiberglass resin. The model was suspended from monofilament lines across the width of the tank and near the substratum, behind the clear partition of the predator compartment. Guppies respond to such predator models and live fish predators in a qualitatively similar manner (29–31, 37).

We quantified the boldness level of paired bright (17.1 \pm 0.3 mm) and drab (16.9 \pm 0.3 mm) males toward the predator model, in both the absence and presence of a female. The experimental protocol was similar to that of the first experiment, except that each pair of males was tested in paired trials

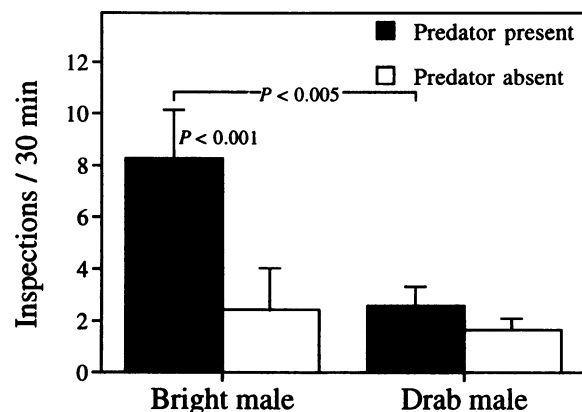


FIG. 1. Mean (\pm SE) number of inspections per 30 min initiated by paired brighter and drabber males toward a live cichlid fish predator ($n = 15$ pairs) or an empty predator tank compartment ($n = 15$ pairs) in the presence of two free-swimming females. Comparisons of boldness scores for paired males within a predator treatment were made using the Wilcoxon test (one-tailed), whereas comparisons of the mean scores of each of the bright and drab males between predator treatments were made separately using the t -test (one-tailed, $df = 28$, data log₁₀ transformed); only comparisons that are statistically significant are denoted with P values throughout.

(presence vs. absence of female) and the duration of a trial was shorter (15 min) to avoid guppy habituation to the model. The order of the paired treatments was determined at random and separated by about 3 h. Each of 24 pairs of males was independently tested in this manner and their respective boldness levels compared. A different stimulus female (21.5 ± 0.6 mm, $n = 24$) was used in each trial.

The frequency of predator inspection visits initiated by each male in the female-present treatment of this experiment was subsequently correlated with the visual conspicuousness of its body color pattern, which was quantified as follows. Following the behavioral tests described above, each male was given an identification number and the conspicuousness of its color pattern was scored blindly of its boldness using a method based on the Munsell color standard matching technique (38) and following in part the methodology of Endler (39). Zuk and Decruyenaere (38) showed that animal color scores obtained using the Munsell method generally correlate with those obtained using Endler's (39) spectroradiometric method, although the latter is more sensitive to detecting variation in color.

Each male was restrained (but not anaesthetized) in a rectangular clear Plexiglas tube ($4.0 \text{ cm} \times 1.5 \text{ cm} \times 1.5 \text{ cm}$), which was immersed into a larger clear Plexiglas canister filled with water and covered at the back with tan paper to provide a uniform background. The apparatus was illuminated overhead with a Sun-Glo fluorescent tube. The right side of the male was then photographed using a 35-mm camera, equipped with a 105-mm 1:1 macro lens and electronic flash, and Kodak Gold 100 ASA print film. While still in his tube, each patch of color (structural colors, carotenoid and melanin pigments) on the right side of the male was scored for its value (lightness or darkness) and chroma (saturation or brightness) by visually matching against standard color chips in the Munsell Book of Color (40). The ranges of possible scores for value and chroma were 2–9 and 2–20, respectively. The area of each color patch and the total area of the male's right side (excluding the dorsal and ventral fins) were measured from its enlarged photograph using a computerized digitizing tablet. The relative area of each color patch was then calculated to control for body size differences among males. Finally, the value scores of all the color patches were added and averaged, and this mean score was then multiplied by the total relative area of all color patches on the male's right side to yield a weighted mean value score. Weighted mean chroma scores were similarly calculated. The sum of the weighted mean value and chroma scores yielded a composite index for the overall conspicuousness of the male's color pattern; higher color index scores correspond to greater visual conspicuousness against a uniform tan background. The color index value calculated for individual male guppies correlated with the relative area of their right side covered with carotenoid pigments ($r_s = 0.31$, $df = 38$, $P < 0.03$; one-tailed, Spearman rank correlation), the latter being a common measure of guppy body coloration pattern used in mate-choice studies (e.g., refs. 10, 11, 21, and 22).

When no female was in view, the boldness of paired bright and drab males toward a standardized predator model were similar (Fig. 2). In comparison, in the presence of a stimulus female, the brightly colored males maintained their high frequency of approaches toward the predator model, whereas the paired drab males reduced their inspections to spend more time near the female (Fig. 2); this resulted in a significant difference in the inspection rates of the paired males toward the predator model, as was similarly observed for the live predator (Fig. 1). There was no treatment order effect; that is, males that were exposed to the stimulus female first and the empty female cylinder second (3 h later) did not behave differently from males that experienced the reverse presentation order ($t = 0.31$, $df = 46$, $P > 0.75$, t -test).

In this second experiment (Fig. 2), there was no correlation between the observed inspection rates of paired males ($r_s =$

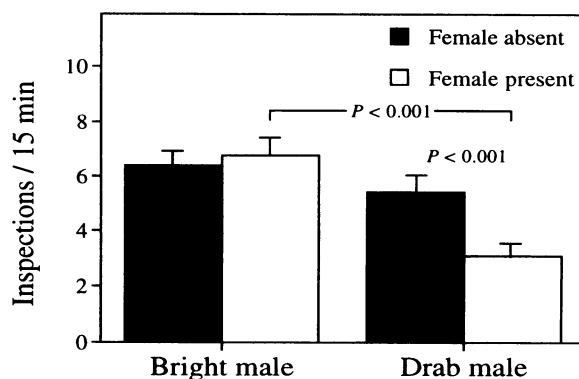


FIG. 2. Mean (\pm SE) number of inspections per 15 min initiated by paired bright and drab males toward a cichlid fish predator model, in either the presence or absence of a female guppy. Mean boldness scores (based on $n = 24$ pairs of males) were compared using the paired t -test (one-tailed, $df = 23$, data \log_{10} transformed); only comparisons that are statistically significant are denoted with P values throughout.

0.089, $df = 22$, $P > 0.80$; Spearman rank correlation), nor between the difference in their respective inspection rates (brighter male - drabber male) and the difference in their body color index ($r = 0.312$, $df = 18$, $P > 0.15$; Pearson correlation). This suggests that the tendency of a given male in a pair to initiate a predator approach in the presence of a female nearby (a common natural situation, see Experiment 4 below) was not strongly influenced by the behavior of the other male. Using the rate of predator inspections initiated by individual males toward a predator model (Fig. 2) as a standardized measure of individual boldness is therefore justified. After statistically controlling for male body length, the boldness level of individual male guppies was significantly positively correlated with their respective body color index (Fig. 3a). That is, the more colorful males approached the predator more frequently than less colorful males.

Experiment 3. If brightly colored male guppies are of higher quality than relatively drabber males then they should be better at escaping predators (cf. ref. 16). One measure of the likelihood of prey escaping from predators is the distance from an approaching predator at which the prey flees (i.e., its flight initiation distance, see ref. 41). In fishes, the risk of a prey being captured is generally greater when it is closer to the predator at the instant of attack (reviewed in ref. 42). This experiment therefore tested the hypothesis that the flight initiation distance of individual male guppies under a simulated predator attack is positively correlated with the conspicuousness of their color pattern.

Males were placed individually in a small clear Plexiglas container, located at one end of a tank ($100 \text{ cm} \times 25 \text{ cm}$, with water 15 cm deep and at $24\text{--}26^\circ\text{C}$), in which they could be enticed by a pair of small parallel mirrors to maintain position at the front of the container and facing in the direction of an approaching cichlid predator model (the same one as in Experiment 2). The tank was illuminated overhead with Sun-Glo fluorescent tubes ($110 \mu\text{E}/\text{m}^2/\text{s}$). The predator model was suspended in the water column (5 cm from the bottom) by two pieces of monofilament nylon lines attached to an overhead ("clothes-line" type) track system. When not moving, the model remained hidden among rocks 60 cm away from the guppy at the opposite end of the tank. The predator model could be moved remotely toward the stationary guppy at 2.5 cm/s (thus simulating a stalking pike cichlid) by activating a stepping motor with a computerized controller. The predator model's stalk began only when the test guppy was stationary at the front of its container and facing the model; the distance separating predator and prey at the beginning of the simulated

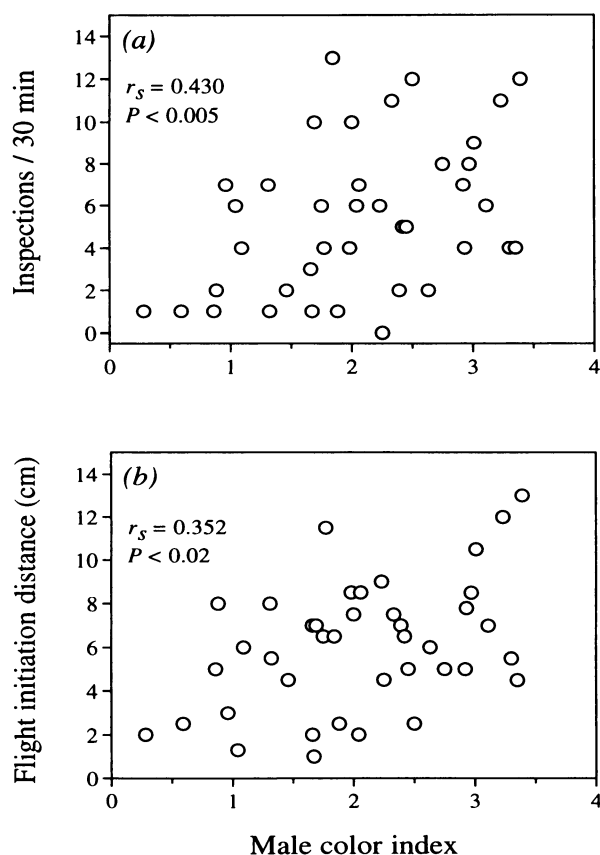


FIG. 3. Relationships between the visual conspicuousness (color index) of the body color pattern of individual male guppies ($n = 40$) and (a) their respective level of boldness, measured independently as the frequency of approaches initiated toward a pike cichlid fish (Experiment 2), and (b) their respective flight initiation distance from an approaching pike cichlid model (Experiment 3). The partial Spearman rank correlation, with fish body length statistically controlled for, and significance value ($df = 37$, one-tailed) are shown for each relationship separately.

stalk was therefore similar for all guppies. Guppy behavior was filmed using an overhead video camera, and the distance separating it and the approaching model at the onset of its escape (rapid flight toward the rear of the container) was measured using a frame-by-frame analysis of the videotape. Forty similar-sized males (the same ones used in Experiment 2) were tested separately, and their flight initiation distances were subsequently correlated with the color index (obtained from Experiment 2) of their respective body color pattern. Males were tested blindly of their body coloration index value.

After statistically controlling for male body length, the distance at which individual male guppies initiated their escape from a "stalking" pike cichlid model was significantly positively correlated with their respective color index (Fig. 3b). That is, the more colorful males fled sooner from the approaching predator, and thus at a greater distance, than less colorful males.

Experiment 4. Female guppies have opportunities to observe males inspecting predators in nature (29, 31). More specifically, in the Quaré River, males and females have been observed (March 1991) to initiate 32% and 68% of recorded inspections ($n = 404$) toward a pike cichlid predator model, respectively; these proportions are not significantly different ($G = 1.72$, $df = 1$, $P > 0.20$, G -test) from those expected based on their relative abundance in the river (35% male, 65% female; J.-G.J.G. and S. A. Davis, unpublished data). In the laboratory ($n = 10$ trials, each using a different group of 2

males and 2 females), Quaré River males made 52% of the recorded inspections toward the same predator model, with the remainder of the inspections made by females and mixed-sex shoals (J.-G.J.G. and S. A. Davis, unpublished data). Given the above and the fact that guppies can recognize individual conspecifics and remember their identity (43, 44), this experiment investigated whether female guppies use observed differences in boldness toward predators among males in making mate choices. To do this, we experimentally uncoupled the observed positive correlation between male boldness and the conspicuousness of their color pattern (see Fig. 3a) in consecutive simulated male predator-inspection trials and female mate-choice trials.

The apparatus for simulating predator inspection behavior consisted of a Plexiglas tank ($67 \text{ cm} \times 20 \text{ cm} \times 24 \text{ cm}$) divided into three adjacent compartments separated by clear Plexiglas partitions. The two smaller compartments ($10 \times 20 \times 24 \text{ cm}$ and $12 \times 20 \times 24 \text{ cm}$), at opposite ends of the tank, housed a live cichlid fish predator (only in the predator-present treatment) and a test female, respectively; they were thus separated by a distance of 45 cm. The larger middle compartment had a Plexiglas track system mounted overhead and along its diagonal, from which two small clear plastic cylinders (33 mm long, 7 mm inside diameter), each containing a male guppy, were suspended via clear plastic rods (2 mm diameter). Because the dimensions of these cylinders closely approximated the body length and depth of the males, the latter could hardly move at all within the cylinders. One of these tubes could be moved remotely along the length of the overhead track, toward and away from the predator compartment, whereas the other tube always remained fixed near (2 cm from) the female compartment. Water was 15 cm deep and maintained at $24\text{--}26^\circ\text{C}$. Overhead illumination was provided with a Sun-Glo fluorescent tube ($28 \mu\text{E}/\text{m}^2/\text{s}$).

A typical simulated predator inspection trial consisted of first placing a virgin female into her end compartment in the tank, and allowing her to acclimate for 2 h with her view of the remainder of the tank blocked by an opaque Plexiglas partition. About 15 min prior to the onset of the trial, one brightly colored male ($16.0 \pm 0.3 \text{ mm}$) was placed in one tube and a similar-sized drabber male ($16.1 \pm 0.3 \text{ mm}$) was placed in the other tube. The order of placement in the tubes was randomized. Initially, the males were placed stationary, 10 cm apart, and broadside 2 cm in front of the female compartment. The opaque partition was then raised and the test female allowed to view (from her compartment) for 10 min the paired males, either in the presence of a live pike cichlid located 45 cm away or in its absence (empty predator compartment). One of the males remained stationary and broadside near the female compartment, and was thus rendered "timid." The other male was rendered "bold" by simulating its inspection of the predator or empty predator compartment, depending on the experimental treatment. A simulated inspection consisted of remotely moving the bold male (with its head always facing in the direction of movement), starting from its broadside position in front of the female, toward the predator compartment in a series three consecutive steps of 10 cm, interspersed by 1-s pauses. After a 3-s pause 5 cm from the predator compartment, the male's cylinder was rotated 180° , moved continuously back to its starting position in front of the female (lasting about 25 s), and turned broadside. Such a simulated inspection visit was repeated six times over the 10-min viewing period.

Immediately following this viewing period, all guppies were removed from the apparatus, placed into a binary mate-choice apparatus, and the female's preference for either of the males tested after a 15-min acclimation period. Female mating preference was assessed in two consecutive 10-min tests, separated by 15 min. In the first test, the males were still in their plastic tubes and thus could not court, whereas they were removed from their tubes and thus could swim freely and court

(with sigmoid displays, see ref. 20) the females in the second test. The binary mate-choice apparatus used and protocol for the mating preference test are similar to those described in Godin and Dugatkin (23). In brief, the apparatus consisted of a female choice arena and two small Plexiglas end chambers, each containing one male, juxtaposed against either end of the choice arena. The sides of the entire apparatus were covered with tan paper to provide a uniform background. During each 10-min test, we recorded the amount of time the female spent near (≤ 10 cm) and facing each male, during which she typically responded to male courtship displays with sexual "gliding" motions (see ref. 20). This time was then expressed as a percentage of the sum of the times she spent near both males. If the female spent more than 55% of her time near (and facing) one of the males, she was considered to have "preferred" that particular male. By this criterion, a preference for a particular male was shown only if the times spent by the female near each of the two males differed by at least 10% (e.g., refs. 23 and 45). The mating preference of individual females determined by such a choice test correlates positively with their choice of mate when actual mating is allowed in the guppy (45, 46), and is consequently used here as an indicator of female mate choice.

Each test female was presented with a different pair of males. The bright male could either be the simulated bold individual and the paired drab male the timid one, or *vice versa*, with treatment order determined at random. Each of these two treatments was replicated with 20 females (18.6 ± 0.3 mm standard length) in both the presence and absence of the predator, for a total of four treatments.

The above experiment was repeated by presenting virgin test females (18.5 ± 0.4 mm, $n = 18$) with similar-sized juvenile stimulus females, instead of paired males, in the tubes in the presence of the predator. The aim of this series of trials was to control for the possibility that a test female might simply choose to associate with any conspecific, irrespective of sex, that she had previously seen (apparently) inspecting a predator nearby.

After having viewed the simulated boldness and timidity of the paired males toward the cichlid predator, female guppies significantly preferred the bold male (inspector) over the timid one (noninspector) as a potential mate, irrespective of their color pattern; this preference was consistent whether the males were still restrained in their plastic tubes and thus not able to court the female (Fig. 4*a*) or free-swimming and able to court (Fig. 4*b*) in the choice tests. However, when the females had previously viewed the males in the absence of a predator, they subsequently preferred the more brightly colored of the paired males, irrespective of the positions of the males relative to the empty predator compartment and whether they were able to court the female (Fig. 4*d*) or not (Fig. 4*c*). Therefore, female guppies preferred to mate with the apparently bolder male (which tend also to be more colorful, see Experiment 2), but only when the threat of predation had been imminent; otherwise, they preferred to mate with the more colorful male regardless of the differences in male activity. Female mate choice was apparently not influenced by male courtship.

When this experiment was repeated with the predator present and with similar-sized and similar-colored females in the tubes, instead of paired males, test females ($n = 18$) showed no preference for either the simulated bold female or the timid female when the latter were still restrained in their tubes (8 vs. 10, respectively; $P > 0.80$, two-tailed binomial test) or when free swimming (8 vs. 10; $P > 0.80$). Therefore, the preferences shown in Fig. 4 are indeed mating preferences and not simply shoaling responses by the females toward the apparently more active males irrespective of the predation or sexual contexts.

DISCUSSION

We have demonstrated for the first time that the visual conspicuousness of the color pattern of male guppies corre-

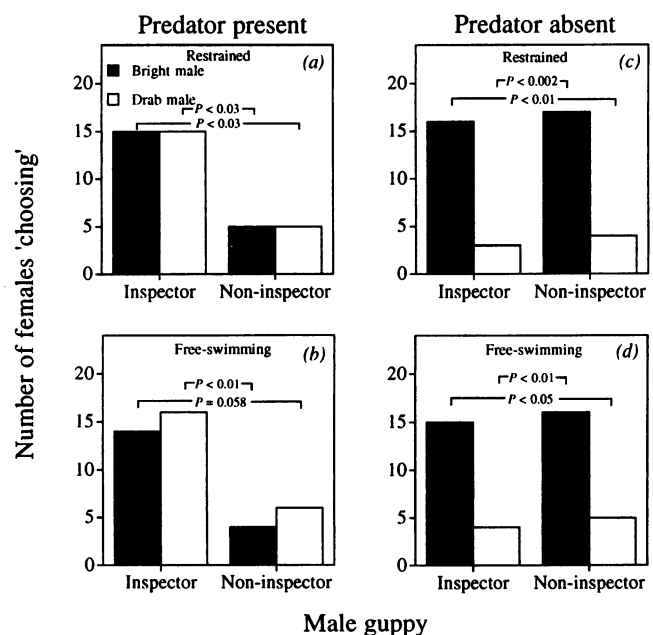


FIG. 4. Mating preferences of female guppies when given a choice between either a bright male inspector (bold individual) and a drab male noninspector (timid individual) or a drab male inspector and a bright male noninspector, whom they had previously observed either in the presence of a live fish predator or in its absence. ($n = 20$ different females for each of the above four mate-choice comparisons; P values shown were obtained using the binomial test, with random choice as the null hypothesis.)

lates positively with their boldness toward a natural fish predator. The respective boldness levels of paired bright and drab males differed only when a threat of predation was imminent (i.e., predator present) and when a female guppy was nearby; the more brightly colored males were significantly bolder than the paired drabber males in both situations. However, the more colorful males did not increase their absolute frequency of predator inspection visits in the presence of a female, as would be expected if they were directly advertising their boldness to females. Nonetheless, brightly colored males were relatively bolder toward the predator and, thus, were apparently willing to incur greater costs (predation risk and lost mating opportunities) than drabber males in the presence of a female, owing to the latter's reduction in predator inspection for greater attentiveness toward the female. Given that cichlid fish predators preferentially attack colorful male guppies (J.-G.J.G. and H. A. Anderson, unpublished data; see also refs. 9, 16, and 19) and that inspection behavior in guppies can deter cichlid predator attack (8), the brightly colored males may have maintained a higher rate of predator inspection than drabber males in the presence of females to counter their greater attractiveness to predators. Therefore, the differential inspection behavior of male guppies in the face of predation hazard potentially provides nearby female guppies information about the males' relative boldness, and perhaps their viability (cf. ref. 8), which they may later use in making mate-choice decisions.

We also found that the visual conspicuousness of the color pattern of male guppies correlates positively with their escape distance from a stalking pike cichlid model predator; that is, the more colorful males fled sooner from the approaching predator, and thus at a greater distance, than less colorful males. Conspicuously colored male guppies may thus be better at escaping fish predators than less colorful ones, as the risk of a prey being captured is generally greater when it is closer to the predator at the instant of attack in fishes (42). This result is novel and as expected if brightly colored male guppies are

at greater risk of being attacked by predators (refs. 9, 16, and 19; unpublished data), and of higher quality (9, 26–28, 45, 47, 48), than drabber males.

It has been previously shown that the amount and brightness of orange color (carotenoid pigments) on male guppies reveals quality, as brightly colored males are more vigorous behaviorally (27, 45, 47, 48) and are likely to be less parasitized (28) and better foragers (9, 26) than drabber individuals. A male's boldness could also reliably indicate his overall quality, as relatively bold fish tend to be better informed about potential predators (6), more likely to survive encounters with them (8), and feed at higher rates or more persistently (refs. 49–51; but see ref. 52) than more timid fish. Bright coloration and boldness in male guppies thus appear to be correlated honest signals of quality, which are difficult to cheat because of their costs (6, 7, 9).

Our study has experimentally shown that Quaré River female guppies can use information about the relative boldness of males whom they have seen interacting with predators in their subsequent mate-choice decisions. They independently preferred colorful and bold males as mates. Such cognitive ability in the guppy has been shown in other behavioral contexts, in which individuals recognized other conspecifics individually, remembered their identity, and subsequently modified their behavior toward each other based on past interactions (43, 44). Although female guppies have opportunities to observe males inspect fish predators (see references and data in the methods of Experiment 4), these opportunities may be rare, because encounters with predators are relatively infrequent in nature (personal observations). Therefore, females likely use male color pattern more often than male boldness as a direct proximate criterion for mate choice, notwithstanding their known use of additional (correlated) cues in assessing potential mates (45, 48). Nonetheless, whenever the opportunity arises, our results suggest that female guppies may use observed differential boldness toward predators among males, rather than the differences in their coloration, in making mate-choice decisions. Boldness may therefore be a more reliable indicator of male viability than body coloration, but the opportunities for its use by females as a proximate cue in mate choice may be limited in nature.

By preferentially mating with colorful males, female guppies are thus choosing on average relatively bold individuals, because these two male traits are positively correlated. In doing so, they may be selecting for highly viable males and, to the extent that viability is heritable, thus gaining indirect fitness benefits by producing offspring with greater viability than otherwise, as proposed by the "good-genes" model of sexual selection (cf. ref. 3). This remains to be demonstrated experimentally. Although bright coloration in male guppies may have evolved as a correlated response to female preferences for such a trait (21, 22, 24), bright male coloration in this species could equally, or in combination, have evolved as a quality-dependent trait selected by adaptive female choice (i.e., good-genes process). The results of the current study and others on the guppy (26–28, 45, 47, 48) are consistent with the latter good-genes process, since they support its basic assumption of a preferred elaborated trait (coloration) in males that is quality-dependent and correlated with viability (see also ref. 53 for additional support using another trait, body size, in male guppies). Further distinction between this and other evolutionary processes of sexual selection (cf. refs. 1–4) in the guppy and in other species will require more knowledge about the fitness consequences of mate choice and the heritability of male viability components, such as foraging ability, boldness, parasite resistance, and antipredator responses.

We thank H. A. Anderson, S. E. Cooper, and S. A. Davis for technical assistance and J. J. Bull, J. A. Endler, P. H. Harvey, A. E. Houde, M. Pagel, and M. Petrie and two anonymous referees for their valuable comments on earlier versions of the manuscript. This research

was supported by the Natural Sciences and Engineering Research Council of Canada and Mount Allison University (grants to J.-G.J.G.) and by the National Science Foundation (a grant to L.A.D.).

- Pomiankowski, A. (1988) *Oxf. Surv. Evol. Biol.* **5**, 136–184.
- Kirkpatrick, M. & Ryan, M. J. (1991) *Nature (London)* **350**, 33–38.
- Andersson, M. (1994) *Sexual Selection* (Princeton Univ. Press, Princeton, NJ).
- Johnstone, R. A. (1995) *Biol. Rev.* **70**, 1–65.
- Ryan, M. J. & Keddy-Hector, A. (1992) *Am. Nat.* **139**, S4–S35.
- Dugatkin, L. A. & Godin, J.-G. J. (1992) *Ann. Zool. Fenn.* **29**, 233–252.
- Wilson, D. S., Clark, A. B., Coleman, K. & Dearstyne, T. (1994) *Trends Ecol. Evol.* **9**, 442–446.
- Godin, J.-G. J. & Davis, S. A. (1995) *Proc. R. Soc. London B* **259**, 193–200.
- Endler, J. A. (1978) *Evol. Biol.* **11**, 319–364.
- Houde, A. E. (1987) *Evolution* **41**, 1–10.
- Houde, A. E. (1988) *Anim. Behav.* **36**, 510–516.
- Norris, K. J. (1990) *Behav. Ecol. Sociobiol.* **27**, 275–281.
- Hill, G. E. (1993) *Biol. J. Linn. Soc.* **49**, 63–86.
- Frischknecht, M. (1993) *Evol. Ecol.* **7**, 439–450.
- Bakker, T. C. M. & Mundwiler, B. (1994) *Behav. Ecol.* **5**, 74–80.
- Endler, J. A. (1980) *Evolution* **34**, 76–91.
- Dugatkin, L. A. (1992) *Behav. Ecol.* **3**, 124–127.
- Götmark, F. (1993) *Proc. R. Soc. London B* **253**, 143–146.
- Endler, J. A. (1991) *Vision Res.* **31**, 587–608.
- Liley, N. R. (1966) *Behaviour Suppl.* **31**, 1–197.
- Houde, A. E. & Endler, J. A. (1990) *Science* **248**, 1405–1408.
- Endler, J. A. & Houde, A. E. (1995) *Evolution* **49**, 456–468.
- Godin, J.-G. J. & Dugatkin, L. A. (1995) *Anim. Behav.* **49**, 1427–1433.
- Houde, A. E. (1994) *Proc. R. Soc. London B* **256**, 125–130.
- Houde, A. E. (1992) *Heredity* **69**, 229–235.
- Kodric-Brown, A. (1989) *Behav. Ecol. Sociobiol.* **25**, 393–401.
- Kennedy, C. E. J., Endler, J. A., Poynton, S. L. & McMinn, H. (1987) *Behav. Ecol. Sociobiol.* **21**, 291–295.
- Houde, A. E. & Torio, A. J. (1992) *Behav. Ecol.* **3**, 346–351.
- Dugatkin, L. A. & Godin, J.-G. J. (1992) *Environ. Biol. Fishes* **34**, 265–276.
- Magurran, A. E. & Seghers, B. H. (1990) *Ethology* **84**, 334–342.
- Magurran, A. E. & Seghers, B. H. (1994) *Behaviour* **128**, 121–134.
- Dugatkin, L. A. & Alfieri, M. (1991) *Evol. Ecol.* **5**, 300–309.
- Magurran, A. E. & Nowak, M. A. (1991) *Proc. R. Soc. London B* **246**, 31–38.
- Seghers, B. H. (1973) Ph.D. thesis (University of British Columbia, Vancouver).
- Tulley, J. J. & Huntingford, F. A. (1987) *Ethology* **75**, 285–290.
- Magurran, A. E. (1990) *Anim. Behav.* **39**, 834–842.
- Godin, J.-G. J. (1995) *Oecologia* **103**, 224–229.
- Zuk, M. & Decruyenaere, J. G. (1994) *Biol. J. Linn. Soc.* **53**, 165–173.
- Endler, J. A. (1990) *Biol. J. Linn. Soc.* **41**, 315–352.
- Munsell (Color Company) (1976) *Munsell Book of Color: Glossy Finish Collection* (Munsell/Macbeth/Kollmorgen, Baltimore).
- Ydenberg, R. C. & Dill, L. M. (1986) *Adv. Study Behav.* **16**, 229–249.
- Godin, J.-G. J. (1997) in *Behavioural Ecology of Teleost Fishes*, ed. Godin, J.-G. J. (Oxford Univ. Press, Oxford), in press.
- Dugatkin, L. A. & Alfieri, M. (1991) *Behav. Ecol. Sociobiol.* **28**, 243–246.
- Dugatkin, L. A. & Sargent, R. C. (1994) *Behav. Ecol. Sociobiol.* **35**, 141–145.
- Kodric-Brown, A. (1993) *Behav. Ecol. Sociobiol.* **32**, 415–420.
- Bischoff, R. J., Gould, J. L. & Rubenstein, D. I. (1985) *Behav. Ecol. Sociobiol.* **17**, 253–255.
- Nicoletto, P. F. (1991) *Behav. Ecol. Sociobiol.* **28**, 365–370.
- Nicoletto, P. F. (1993) *Anim. Behav.* **46**, 441–450.
- Murphy, K. E. & Pitcher, T. J. (1991) *Ethology* **88**, 307–319.
- Wilson, D. S., Coleman, K., Clark, A. B. & Biederman, L. (1993) *J. Comp. Psychol.* **107**, 250–260.
- Godin, J.-G. J. & Crossman, S. L. (1994) *Behav. Ecol. Sociobiol.* **34**, 359–366.
- Magurran, A. E. (1993) in *Behaviour of Teleost Fishes*, ed. Pitcher, T. J. (Chapman & Hall, London), pp. 441–477.
- Reynolds, J. D. & Gross, M. R. (1992) *Proc. R. Soc. London B* **250**, 57–62.